Milk leptin in sows and blood leptin and growth of their offspring[1,2]

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ABSTRACT: Twenty-one mixed-parity (average 2.4 ± 0.49) crossbred sows and their offspring were used to determine whether sow milk leptin at farrowing was related to neonatal serum leptin and pig growth to weaning. During farrowing, pigs were randomly allotted to suckling (n = 99) or delayed suckling (n = 89) groups, with delayed suckling pigs placed in a group pen apart from the dam before suckling. Both groups had access to heat lamps. Colostrum samples were collected no more than 2 h after farrowing the first pig. Blood samples were collected from all pigs approximately 2 h after farrowing was complete; pigs were then ear notched and returned to their dams. Pig BW was recorded at 1.2 ± 0.04 d of age and again at weaning. Milk and blood serum were collected after centrifugation; leptin concentrations were estimated using RIA. Leptin was detected in colostral milk, as expected, and averaged 46.0 ± 1.1 ng/mL. Pig serum leptin ($P < 0.02$) was greater in suckling pigs than in delayed suckling pigs, averaging 0.69 ± 0.08 and 0.54 ± 0.07 ng/mL, respectively. Although male pigs were heavier ($P < 0.01$) at birth than female pigs (1,507 ± 52 vs. 1,381 ± 43 g), ADG to weaning and weaning weights were similar for both sexes, averaging 229 ± 14 g and 5,829 ± 323 g, respectively, for all pigs; serum leptin concentrations were not affected by sex of the pig. Milk serum leptin was not associated with litter size, parity, pig birth weight, ADG to weaning, or weaning weight. Suckling status did not influence ADG to weaning or weaning weight of pigs; neonatal pig serum leptin was not related to birth weight, weaning weight, or ADG to weaning. These results indicate that leptin is not directly related to early neonatal growth in the pig; however, more in-depth studies are needed to determine possible indirect or long-term effects of early leptin exposure.

Key words: growth, leptin, milk, pig, relationship, sow

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INTRODUCTION

Although leptin is secreted from other tissues, it is produced primarily by white adipose tissue, which also contains the leptin receptor. In the pig, along with adipose tissue, expression of the leptin receptor has been found in the hypothalamus, anterior pituitary gland, ovary, uterine body, liver, kidney, pancreas, adrenal gland, heart, spleen, lung, intestine, bone marrow, and muscle (Lin et al., 2000). Therefore, it is possible that leptin can influence a variety of physiological functions. Leptin has also been found in the placenta (Hoggard et al., 1997; Ashworth et al., 2000), where it passes through to the fetus during its transition to the neonate (Matsuda et al., 1999), so both maternal and fetal concentrations of leptin are increased during pregnancy. Leptin is also produced by the mammary gland, and has been found in the milk of several species, including goats (Whitley et al., 2005), sheep (McFadin et

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al., 2002), pigs (Estienne et al., 2000), horses (Salimei et al., 2002), cattle (Wathes et al., 2007), and humans (Houseknecht et al., 1997). Additionally, in a review of obesity in humans and early life programming, Cottrell and Ozanne (2007) confirmed a small protective effect of breast-feeding against later obesity, and suggested that early ingestion of leptin in milk by the neonate might influence later growth and development. Little research has been conducted to determine the relation of leptin in pigs to preweaning growth. Therefore, the objective of this experiment was to investigate possible relationships among sow milk and pig serum leptin and subsequent pig growth to weaning.

MATERIALS AND METHODS

All animal-related procedures were approved by the University of Maryland Eastern Shore Institutional Animal Care and Use Committee.

Animals and Procedures

Twenty-one mixed-parity (average 2.4 ± 0.49) crossbred sows (PIC × Landrace × Yorkshire) and their offspring at the University of Maryland Eastern Shore were used during May to July of the first year. After AI using pooled semen from 2 crossbred boars, sows were housed in standard gestation stalls in a commercial-type, curtain-sided swine facility on a natural light cycle. Sows had ad libitum access to water and were fed a commercially pelleted, 14% CP gestation ration with a minimum of 3% crude fat (14% Warm Environment Gestation ration, Southern States Inc., Gettysburg, PA) at approximately 2.7 kg/d until after farrowing. Sows were moved to environmentally controlled rooms with a 12:12 light:dark cycle approximately 7 d before the expected date of farrowing for the first animal mated. Sows were observed during the farrowing process, and a colostrum sample was obtained within 2 h of farrowing but after farrowing at least one pig. In the next feeding period after farrowing (within 12 h), sows were switched to a commercially pelleted, 16% CP lactation feed that was offered on an ad libitum basis (16% Mega Sow Lactation, Southern States Inc.). An average of 11.5 ± 0.7 pigs were born live per sow (total litter size of 12.3 ± 0.2, including stillborns); however, only apparently healthy, phenotypically normal pigs that were observed being delivered were used (n = 188; 9.0 ± 0.7 pigs/sow).

During farrowing of each sow, pigs were randomly allotted to groups of suckling (n = 99) or delayed suckling (n = 89). Suckling pigs were left with the dam with access to a heat lamp, whereas delayed suckling pigs were placed in a group pen with a heat lamp apart from the dam in the same room for a maximum of 8 h. Approximately 2 h after the end of farrowing (to allow all suckled pigs to ingest colostrum), blood samples were collected from an anterior vena cava from all pigs on the study (1 mL), placed into 12 × 75 mm borosilicate glass tubes without additive (VWR, Bridgeport, NJ), sex of the pig was recorded (40 male, 59 female suckled; 44 male, 45 female unsuckled), and pigs were ear-notched for permanent identification. After sampling, all pigs were returned to sows, with no cross-fostering conducted. Blood samples were allowed to clot at 4°C overnight and serum was collected after centrifugation at 2,500 × *g* at 4°C for 15 min. Milk samples were stored at −20°C until being ultracentrifuged at 100,000 × *g* at 5°C for 1 h. The clear supernatant (milk serum) was removed and stored at −20°C until analysis. Leptin concentrations in blood serum and milk serum were measured using the leptin RIA described by Delavaud et al. (2000) and previously validated for use with milk serum (McFadin et al., 2002). Inhibition curves generated by dilutions of pig serum from 25 to 350 μL were parallel to the standard curve.

At 1.2 ± 0.04 d of age, pigs were weighed and processed (100 mg of iron dextran injected i.m., needle teeth and tails clipped, males castrated). Pigs were weaned at 23.8 ± 0.31 d of age, and BW and number weaned were recorded. Average daily gain was calculated as grams per day of BW gain. Stillbirth and preweaning mortality BW were recorded up to the date of pig processing.

Statistical Analysis

Data were analyzed using mixed model least squares procedures (SAS Inst. Inc., Cary, NC) with $P < 0.05$ considered significant. The initial linear model used in the analyses of individual pig data included the fixed effects of parity, treatment, sex, weaning age (linear), all possible interactions among fixed classification effects, and the random effects of sow within parity and the pooled random interaction of sow within parity with fixed effects. Sow was cross-classified with treatment and sex, and sow was the main unit experimental unit. Pigs within sow and treatment were the subunit experimental unit.

Models were reduced in a step-wise procedure by elimination of unimportant 3-factor fixed interactions ($P > 0.25$) and 2-factor fixed interactions, in that order. In accordance with appropriate model reduction procedures, factors (main effects and lesser order interactions) included in significant interactions ($P < 0.25$) were left in the linear model independent of their level of significance. Additionally, estimation of subclass means required inclusion of interactions in the linear model for estimability. Parity effects were tested by sow within parity, and other fixed effects were tested by the pooled error of sow within parity interactions with fixed effects.

The relationships of pig serum leptin concentrations to birth weight, preweaning ADG, and weaning weight were analyzed by including serum leptin in the above linear mode, with model reductions performed as described previously. The initial linear model used in the analyses of sow data included the fixed effects of parity,

Table 1. Least squares means (±SEM) for variables measured in male and female pigs allowed to suckle from the dam (suckling) or not suckle from the dam (delayed suckling) until 2 h after farrowing was complete

	Pig sex and suckling status			
Variable	Male (n)	Female (n)	Delayed suckling (n)	Suckling (n)
Serum leptin, ng/mL	0.61 ± 0.08 (77)	0.62 ± 0.07 (89)	0.54 ± 0.07[a] (85)	0.69 ± 0.08[b] (81)
Birth weight, g	1,507 ± 52[x] (84)	1,382 ± 43[y] (104)	1,424 ± 45 (89)	1,466 ± 45 (99)
ADG to weaning, g	228 ± 15 (59)	230 ± 13 (68)	238 ± 13 (62)	220 ± 13 (65)
Weaning weight, g	5,872 ± 343 (59)	5,785 ± 303 (68)	5,991 ± 303 (62)	5,667 ± 305 (65)

[a,b]Within suckling and row, means without a common superscript differ ($P < 0.02$).
[x,y]Within sex and row, means without a common superscript differ ($P < 0.01$).

treatment, parity × treatment, and weaning age (linear) and the random effects of sow within parity and sow × treatment within parity. Model reductions were performed similarly to the procedure given for the analyses of individual pig data. The relationships of sow milk leptin concentrations to birth weight, preweaning ADG, and weaning weight were analyzed by including milk leptin in the above linear model as a covariate, with model reductions performed as described above.

RESULTS AND DISCUSSION

In the present study, leptin was found in the colostral milk serum of sows (averaging 46.0 ± 1.1 ng/mL), and pig serum leptin was greater ($P < 0.02$) for suckling than for delayed suckling pigs (Table 1). Colostral milk was reported to have increased concentrations of leptin in other studies with pigs (Estienne et al., 2000) as well as for other species, including meat goats (Whitley et al., 2005), sheep (McFadin et al., 2002), and mares (Salimei et al., 2002; Berg et al., 2007). The increased concentrations of leptin in periparturient milk was theorized to be attributed to a pooling of leptin in the udder before parturition in sheep (McFadin et al., 2002), although Rasmussen et al. (2008) indicated that prepartum accumulation of leptin in colostrum did not seem to occur in dairy goats (n = 4).

Overall, it is accepted that relatively increased concentrations of milk leptin occur at parturition, which coincides with the time when neonates are best able to absorb large proteins through the gastrointestinal tract, and evidence does exist that the neonate can absorb leptin ingested orally. For example, as with the present study, elevated blood serum leptin was found in neonatal suckled compared with unsuckled rat pups (Dessolin et al., 1997). Blood leptin was greater in rat pups fed milk plus leptin vs. milk alone (Casabiell et al., 1997), in neonatal pigs suckled vs. being fed milk replacer (Weiler et al., 2002), in neonatal calves fed colostrum vs. milk replacer (Blum et al., 2005), and in breast-fed compared with formula-fed human infants (Savino et al., 2004). In addition, Sànchez et al. (2005) noted that leptin is absorbed through the neonatal stomach in rat pups, and Berg et al. (2007) noted that leptin concentrations in neonatal foals increased significantly after nursing. In contrast, goat kids removed from the dam at birth and bottle-fed colostrum within 3 h after birth did not have greater concentrations of serum leptin at 6 h after feeding than before colostrum feeding (Rasmussen et al., 2008). Therefore, although leptin certainly has the potential to play a role in the development of the neonate either systemically or locally at the level of the gastrointestinal tract, more intensive studies are needed.

Pig serum leptin was not related to birth weight in this study. Because neonatal pigs have decreased amounts of adipose tissue in general and lack brown adipose tissue (Herpin et al., 2002), the well-documented positive relationship between body fat (especially brown adipose tissue) and serum leptin concentrations might explain this result. In agreement with the present study, lamb birth weight was independent of body fat and was thus independent of plasma leptin concentrations (Ehrhardt et al., 2003). In humans, a species with adequate neonatal adipose tissue, cord serum leptin at birth was positively correlated with birth weight (Tamura et al., 1998; Perrone et al., 2000; Martinez-Cordero et al., 2006) but was not independent of percentage of body fat (Martinez-Cordero et al., 2006). Pig serum leptin at birth was also not significantly related to weaning weight or ADG in the present study. Because pigs with delayed suckling were not offered liquids during separation and were placed back on their dams within 8 h of removal, ingestion of some colostrum (and thus some leptin) could still have occurred. In other species, colostral leptin decreased quite dramatically by 12 h after parturition (McFadin et al., 2002; Whitley et al., 2005; Berg et al., 2007), which could point to relatively negligible concentrations being ingested by the pig in the delayed suckling group if the same were true for sows, but milk serum leptin has not been measured in frequent intervals after parturition in this species. However, because suckling status did not influence ADG to weaning or weaning weight of pigs (Table 1), the delayed ingestion of leptin would likely not have affected the results of the study.

Milk serum leptin was not associated with litter size, pig birth weight, ADG to weaning, or weaning weight. Breast milk leptin was also not related to infant body mass in humans (Uysal et al., 2002), and in goats, milk serum leptin throughout lactation was not associated with kid BW when measured from birth to 21 d of age or from 7 to 56 d of age (Whitley et al., 2005).

Suckling status did not influence the average number of pigs weaned per sow (8.6 ± 0.2). The unusually increased mortality rates were primarily due to crushing, which was thought to be caused by increased agitation of sows resulting from the novel human interactions required for the study and the use of several first-parity sows (gilts).

Although male pigs were heavier ($P < 0.01$) at birth than female pigs, ADG to weaning and weaning weights were similar for both sexes, and sex was not associated with serum leptin concentrations (Table 1). There was also no difference in cord blood leptin concentrations between male and female human neonates (Okereke et al., 2002), and there was no influence of sex (castrated males vs. females) on circulating leptin concentrations in lambs, although serum leptin and BW were measured over a 47-d period after birth (McFadin et al., 2002) compared with only at birth for the pig and human studies.

In the current study, no apparent relationship between dam milk leptin and offspring growth was noted. In addition, regardless of suckling status, no relationship was noted for pig serum leptin and growth to weaning. Therefore, as seen in lambs (McFadin et al., 2002) and goats (Whitley et al., 2005), the present data are consistent with the current lack of convincing evidence for leptin as a direct modulator of neonatal growth. However, the long-term effects of milk leptin and the possible indirect effects of milk leptin on neonatal development, survival, or other factors not measured in this study, such as those theorized for neonatal gut development (Wolinski et al., 2003), or on thermoregulation (Stehling et al., 1997, Mistry et al., 1999, Mostyn et al. 2002) as discussed previously (Whitley et al., 2005), should be studied further.

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